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REVIEWS

Sequence-Specific DNA Minor Groove Binders. Design and Synthesis of Netropsin and Distamycin Analogues

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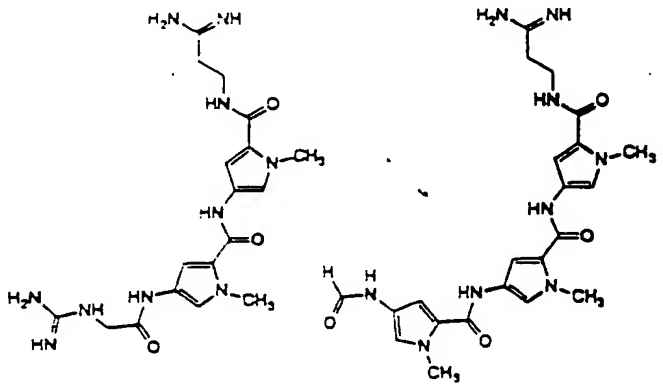
Over the past two decades, impressive advances have occurred in the understanding of how a normal cell transforms into a tumor cell. Molecular and cellular studies have shown that point mutations, deletions, translocations, and other types of rearrangements in DNA affect either the expression or the biochemical function of specific genes, be it an oncogene or a tumor-suppressing gene. By designing a drug capable of recognizing a specific sequence in DNA, it may be possible to specifically inhibit the expression of certain oncogenes and thereby to control the development of tumor cells. The message provided by the molecular biologist is that specific DNA sequence changes can switch on the malignant transformation of a cell. The goal of the pharmacologist is to find a drug to switch it off.

The possibility that low molecular weight ligands might bind to specific sequences in DNA was raised long ago (*1*, *2*). The antiviral antibiotics netropsin (Net) and distamycin (Dst) were arguably the first drugs discovered that bound selectively to AT-rich sequences in the minor groove of DNA. So far, >20 high-resolution structures obtained by NMR and X-ray crystallography have been reported for a variety of oligonucleotide sequences complexed with netropsin, distamycin, and related minor groove binders (Table 1) (*3*–*19*). Quantitative footprinting methods have been used to analyze the sequence



netropsin distamycin

preference (*20*–*25*). Computational studies have contributed information that provides a rational explanation for the selective fit of these crescent-shaped ligands into the minor groove of DNA (*26*, *27*). Thermodynamic studies of Net and Dst binding to DNA, and their effects on the formation of protein/DNA complexes, have also been thoroughly investigated (*28*–*40*). Since the pioneer discoveries of the AT-selective binding of Net and Dst to DNA (*41*), a considerable number of physicochemical, biochemical, and biological studies have been reported to increase our understanding of the details of the mechanisms by which these two antibiotics bind to and recognize double-stranded DNA squence (*42*). This detailed information has been exploited by medicinal chem-

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ists to develop molecules capable of reading specific DNA sequences and, most importantly, to establish molecular rules for the design of sequence recognition elements. Although studies are still in progress, it is already clear that the design of sequence-specific ligands promises to be one of the great success stories of pharmacology. A brief survey of the story is reviewed here. This review is mainly concerned with the different categories of netropsin and distamycin derivatives synthesized during the past 15 years.

1. GENERAL MECHANISM OF GROOVE BINDING

The general mechanism of groove binding is shown schematically in Figure 1. Conceptually, the binding process may be divided into at least two parts. First, the groove binding agent undergoes a hydrophobic transfer from solution into the DNA minor groove. If the groove binder is positively charged, this event will be accompanied by the release of condensed counterions that surround the DNA. Once in the minor groove, specific molecular interactions may then form, including van der Waals attraction and the formation of hydrogen bonds.

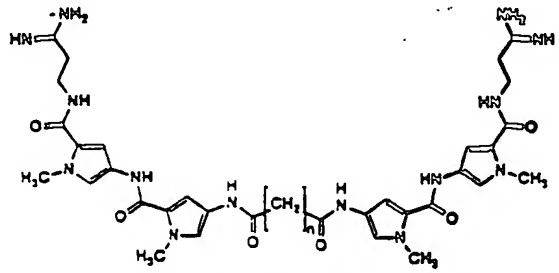
The detailed energetics of groove binding reactions have yet to be fully elucidated, but the following elements are minimal contributions that must be considered. The formation of any bimolecular complex contains a substantial unfavorable free energy contribution resulting from the loss of rotational and translational degrees of freedom as two reactants form a single complex. Other, energetically favorable, contributions must then balance and overwhelm this entropic cost of forming the complex. The favorable contribution from the hydrophobic transfer process to the free energy is expected to be large and may largely balance the entropic penalty. Smaller, but no less important, favorable contributions come from the polyelectrolyte contribution to the free energy, arising from counterion release and from the formation of the various noncovalent molecular interactions. Whereas such molecular interactions are usually very much the focus of high-resolution structural studies, it must always be kept firmly in mind that such interactions are the culmination of a complex, multistep reaction mechanism. The stability of the final complex arises from an often delicate balance of favorable and unfavorable free energy contributions at each step along the reaction pathway.

2. THE LEXITROPSINS: MONOMERS AND DIMERS

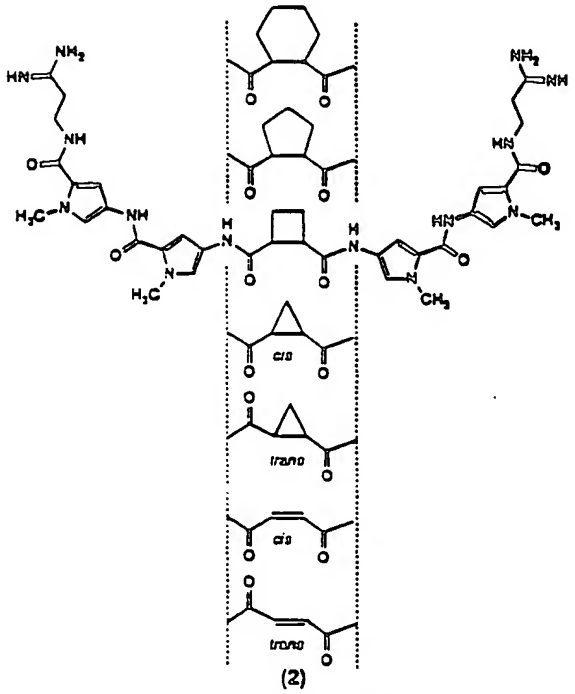
Three main factors are thought to contribute favorably to the stability between AT sequences and netropsin or distamycin: (i) hydrogen bonding between the amide NH of the drug and thymine O-2 and adenine N-3 atoms; (ii) an overall shape complementarity resulting in close ligand–DNA van der Waals contacts; and (iii) polyelectrolyte interactions between the polyanionic DNA and the cationic drugs. It is assumed that the van der Waals interactions are pivotal in the sequence recognition (*43*).

The first design strategy employed to generate new compounds was to extend the length of the groove binding agents in hopes of extending the length of the binding site. Tris, tetra, penta, and hexa *N*-methylpyrrolecarboxamide derivatives of Net and Dst can bind to sequences containing five, six, seven, and eight contiguous A·T base pairs, respectively. The binding site size for an analogue containing $n - 1$ pyrrole rings or n amide bonds is $n + 1$ base pairs long in terms of occluding base pairs (or n in terms of contacted base pairs) (*44*, *45*). This rule is valid for analogues containing up to six *N*-methylpyrrolecarboxamide residues but not for longer molecules. A hepta *N*-methylpyrrolecarboxamide derivative does not fit very well with the natural twist of the DNA, presumably because the molecule gets out of phase with the base pairs along the minor groove floor of the double helix. Indeed, the pyrrolecarboxamide unit is ≈20% longer than required to match perfectly the base pair rise in the minor groove (*46*). Two alternative strategies have been envisaged to circumvent the poor phasing between the DNA and *N*-methylpyrrolecarboxamide-containing ligands.

The first consists of joining two netropsin or distamycin molecules by a linker of suitable length to permit bidentate binding to DNA. A considerable number of bis(netropsin)s containing different types of linkers have been elaborated (*47*–*51*). Bis(netropsin)s coupled by a polymethylene tether (**1**) can engage in bidentate binding



flexible bis-netropsins (**1**)
n= 1-10



(**2**)

rigid bis-netropsins

providing that the connector contains at least three methylene groups. However, owing to the flexibility of the aliphatic connector, monodentate binding of such bis(netropsin)s is also possible. In contrast, bis(netropsin)s possessing conformationally rigid linkers (**2**) can readily bind to sequences containing 8–10 consecutive AT base pairs without the unwanted monomer binding to shorter sequences (*52*, *53*). For chiral bis(netropsin)s, the stereochemistry of the linker is critical and may be used for controlling the directionality in binding to DNA (*54*). Recently, two bis(distamycin) derivatives possessing a 3,5-*m*-pyridyl or a *trans*-vinyl linker were found to be the

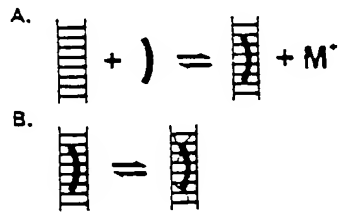
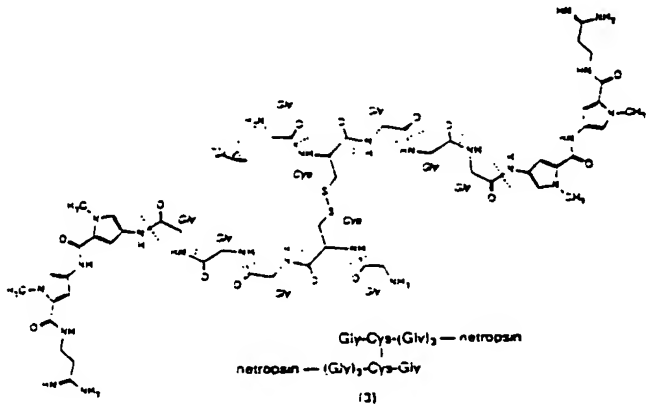


Figure 1. Schematic representation of hypothetical steps in the reaction pathway from DNA groove binding: (A) A groove binding agent is hydrophobically transferred from solution into the minor groove. If the ligand is positively charged, condensed counterions territorially bound to the DNA will be released. (B) Once in the minor groove, the ligand can form a variety of molecular interactions, including hydrogen bonds and van der Waals attractions.

Table 1. Structures of Oligonucleotide/Netropsin and/or Distamycin Complexes Determined to Atomic Resolution

drug/DNA complex	reference
netropsin/d(CGCGAATTCGCG)$_2$	3
netropsin/d(CGCGAATT^{5Br}CGCG)$_2$	4, 5
distamycin/d(CGCGAATTCGCG)$_2$	6
distamycin/d(CGCAAATTTGCG)$_2$	7
netropsin/d(CGCGATATCGCG)$_2$	8
2 distamycin/d(CGCAAATTGGC)	9, 10
netropsin/d(CGC[e^{6}G]AATTCGCG)	11
netropsin/d(CGCAAATTTGCG)$_2$	12
2 distamycin/d(CGCAAGTTGGC)	13
netropsin/d(CGCGAATTCGCG)$_2$	14
distamycin/d(ICICICIC)$_2$	15
distamycin/(IcIcICIC)$_2$	16
2 distamycin/d(ICITACIC)$_2$	17
2 distamycin/d(ICATATIC)$_2$	17

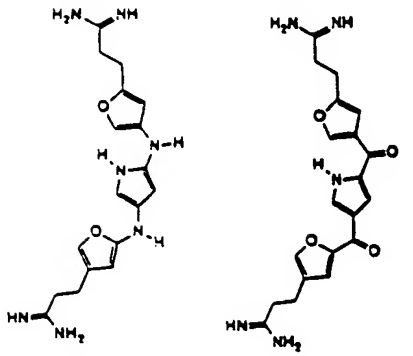
most effective bis-linked lexitropsins at inhibiting transcription by HIV-1 reverse transcriptase (*55*). The linker can also play an active role in the DNA recognition process. For example, the bis(netropsin) analogue 3, in



(3)

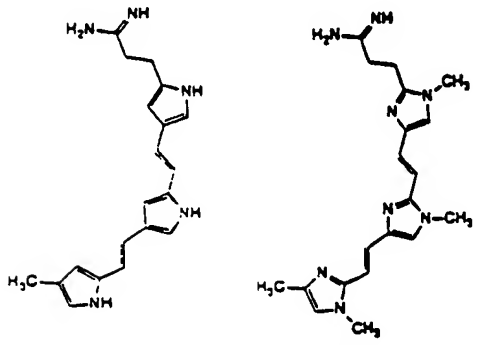
which the two netropsin residues are connected by a disulfide bond between two Gly-Cys-Gly peptides, binds more strongly to sequences containing a central GC step such as (AT)$_3$(GC)$_2$(AT)$_3$ than to strictly homologous (AT)$_n$ sequences (*56*, *57*).

The second strategy consists of replacing the carboxamide bond in netropsin and distamycin with shorter keto or amino linkages. Molecular modeling predicts that isohelical molecules such as 4 and 5 (called isolexins) would bind more tightly in the minor groove than their corresponding carboxamide analogues (*46*, *58*). As for the lexitropsins (see below), the use of imidazole or furan rings would favor recognition of GC sequences. In the same way, the computational studies predict that the replacement of the carboxamide bond of netropsin with an ethylene bond (compound 6) would permit an optimum



(4) (5)

isolexins



(6) (7)

vynilexins

fit to the minor groove surface (*59*). According to the computational measurements, molecules called vinylexins containing both −C=C− linkages and imidazole and or furan heterocycles (e.g., compound 7) would display a considerable preference for GC-rich sequences (*60*). Although these suggestions are very promising, so far neither the isolexin nor the vinylexin strategy has been experimentally tested.

The guanine 2-amino group protrudes into the minor groove and obstructs the access of drugs to the floor of the groove. The fact that the 2-amino group constitutes a critical negative recognition element for binding of small molecules, including Net and Dst, in the minor groove of DNA has now been unambiguously demonstrated using DNA molecules in which that group has been either deleted from guanines and/or added to adenines (*61*−*65*). Given both the strategic position of the guanine 2-amino group in the minor groove and its hydrogen bonding capacity (it is the only H bond donor exposed in the minor groove), it was proposed that the introduction of an H bond acceptor heteroatom in the pyrrole rings of netropsin might permit the drug to bind to GC sequences (*46*). Lown and co-workers have extensively exploited this concept and synthesized an impressive number of molecules christened "lexitropsins" of information reading oligopeptides structurally related to netropsin and distamycin (*66*, *67*). By substituting imidazole, thiazole, triazole, pyrazole, or oxazole heterocycles for the *N*-methylpyrrole rings of netropsin, one can design drugs capable of binding to sequences containing one or two G·C pairs embedded in an AT sequence (Figure 2). Among the numerous lexitropsins synthesized so far, imidazole lexitropsins such as compounds 8−10 display the most pronounced capacity for binding

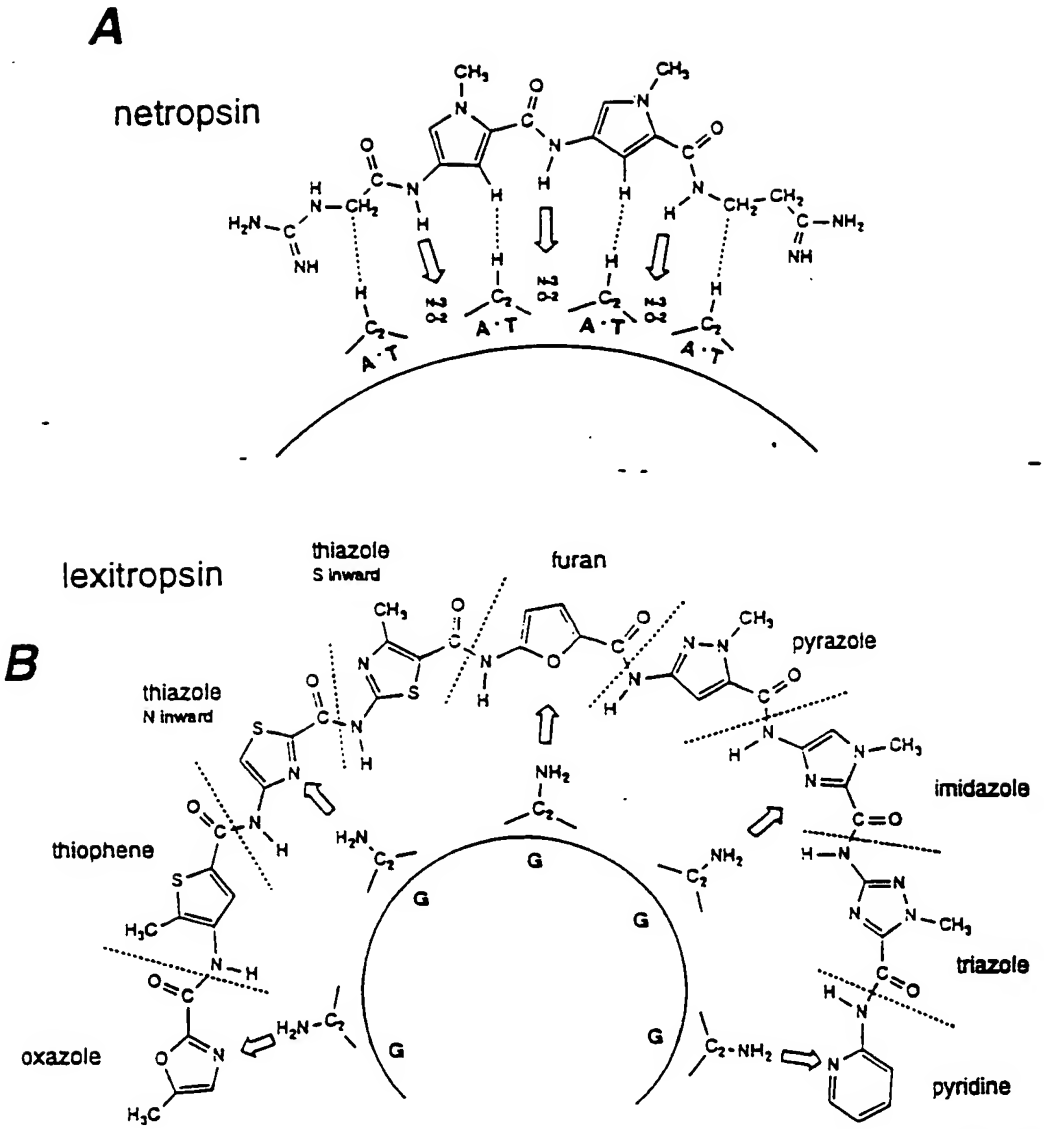
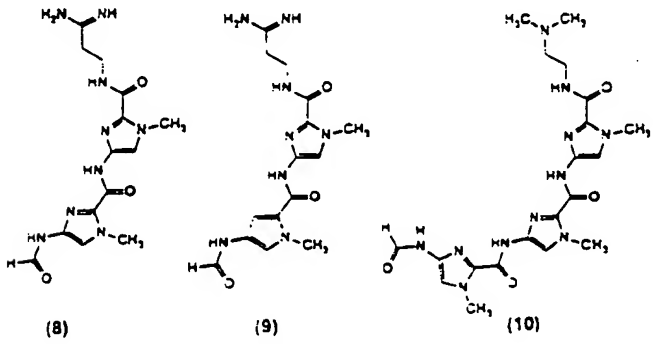
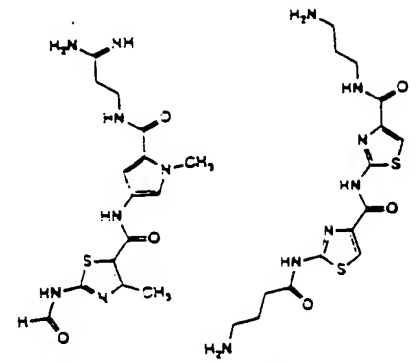


Figure 2. (A) Representation of the binding to DNA of netropsin: (B) proposed representation of a model lexitropsin molecule with guanine residues in DNA. Heavy arrows are hydrogen bonds, from donor to acceptor. Dashed lines mark close van der Waals nonbonded contacts between DNA and drug.



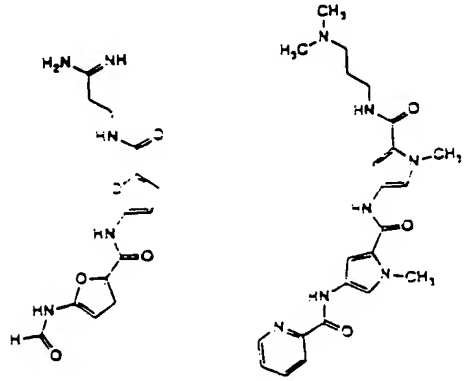
imidazole-lexitropsins

to GC-containing sequences (*68*–*79*). Thiazole lexitropsins either accept or avoid a G·C base pair in their binding sites, depending on the position of the sulfur atom (*80*–*83*). For example, the lexitropsin **11** with the sulfur atom directed into the minor groove does not bind to GC-containing sites, whereas the lexitropsin **12** containing two thiazole rings with the sulfur atoms directed outward from the minor groove binds best to alternating purine–pyrimidine sequences such as 5′-TATGAC and 5′-TGCATGC (*84*). The same type of result was obtained with the furan-containing lexitropsin **13** (*85*). To summarize, the lexitropsin approach based on a 1:1 complex has led to minor groove binders with increased tolerance for G·C base pairs in the binding site but did not lead to the design of a purely GC-specific molecule. According to computational studies, the observation that most lexitropsins can accommodate both AT- and GC-containing sequences comes in part from the fact that the electrostatic potential in the minor groove of AT-rich regions is very negative (*86*). The electrostatic interactions between AT sites and the positively charged end groups in the lexitropsins presumably provide the initial attraction. The binding of mono- and dicationic minor groove binders to AT-rich regions has a significant electrostatic component (*87*–*89*). However, the fact that neutral lexitropsins show the same interaction with AT sequences as the monocationic distamycin tripeptide argues against the dominant role of electrostatics in sequence selectivity (*90*). A recent comprehensive spectroscopic and thermodynamic study of the interaction of the minor groove binder Hoechst 33258 with the d(CG-CAAATTTGCG)$_2$ duplex suggested that the hy[illegible]ic



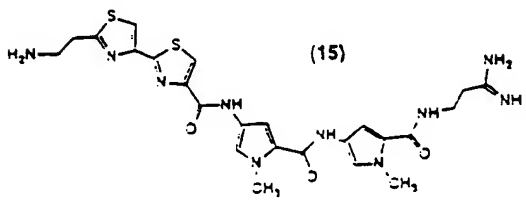
(11) (12)

thiazole-lexitropsins



(13) (14)

furan-lexitropsin pyridine-lexitropsin

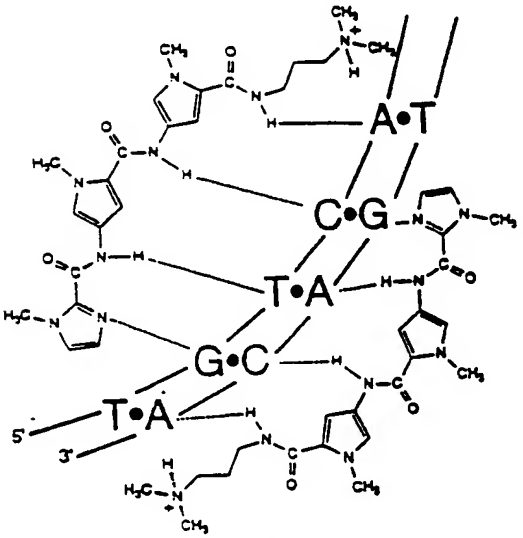


(15)

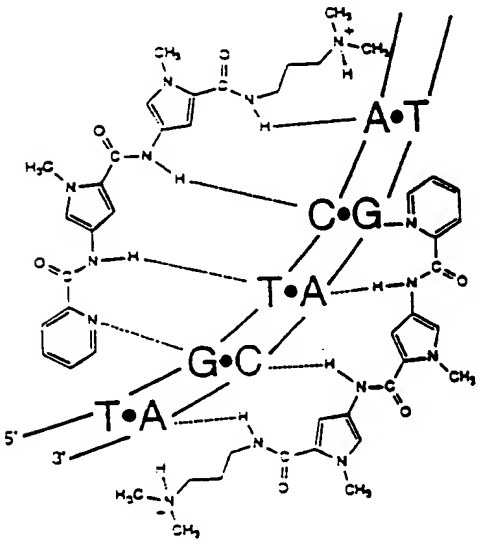
transfer of the drug from solution into the duplex binding site provides the major driving force for the binding reaction (*91*).

An alternative approach to design minor groove binders capable of binding GC pairs is to connect a netropsin-like molecule with GC-recognizing elements. For example, substitution of the terminal formamido-methylpyrrole group of distamycin for a pyridine group (compound 14) permits interaction with a G-C base pair. However, as for the imidazole lexitropsin, the binding to pure AT sequences is not abolished (*92*, *93*). The bithiazole moiety of the antitumor drug bleomycin has offered opportunities for binding to GC sites. The netropsin moiety of the conjugate 15 drives the drug to AT sites and allows the appended bithiazole unit to contact a pyrimidine–G–pyrimidine motif (*94*).

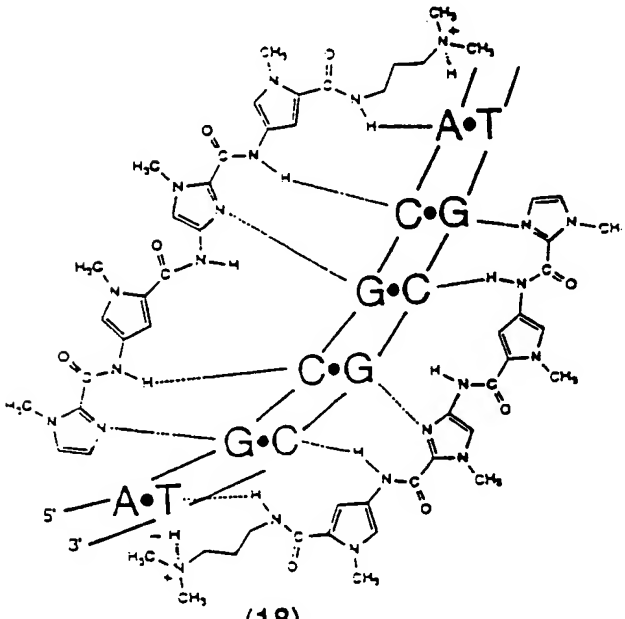
The discovery in 1990 that the minor groove of DNA can expand slightly to accommodate two distamycin molecules associated side-by-side in an antiparallel head-to-tail orientation has rekindled interest in the design of lexitropsin compounds (*9*, *10*, *15–17*, *95*). The 2:1 drugs/DNA motif has been observed with different distamycin analogues including a carbamoyl tetrapyrrole derivative (*96*). This landmark discovery immediately inspired the design of homo- and heterodimeric ligands. Imidazole- and pyridine-containing lexitropsins (compounds 16 and 17) were combined side-by-side so as to



(16)

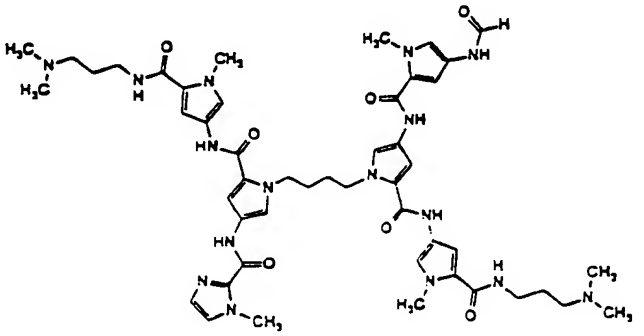


(17)

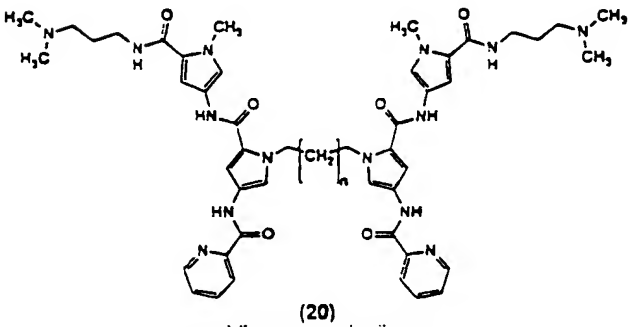


(18)

permit direct interaction with G·C pairs via hydrogen bonding with the 2-amino group of guanines (*13*, *97*–*99*). The imidazole-containing homodimer (**18**) binds specifically to GCGC sequences. This was the first instance of a minor groove binding lexitropsin being directed uniquely to GC sites (*100*). Combination of side-by-side pyrrole and imidazole rings permits G·C base pairs to be differentiated from C·G base pairs (*101*). The sequence selectivity, affinity, and geometry of the drug/DNA 2:1 complex can be optimized by choosing appropriate pairs of ligand molecules with complementary recognition properties and by covalently linking the two DNA reading elements (compounds **19** and **20**) (*102*–*111*).



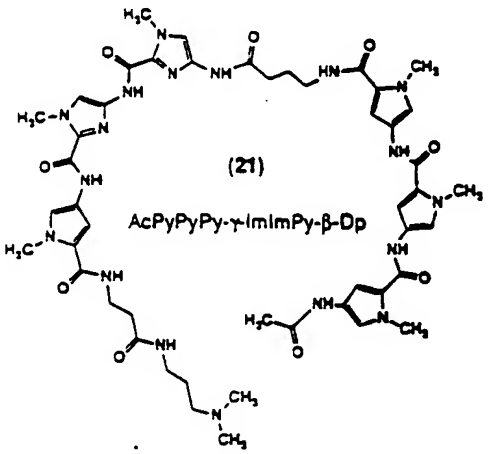
(19)
imidazole-netropsin dimer



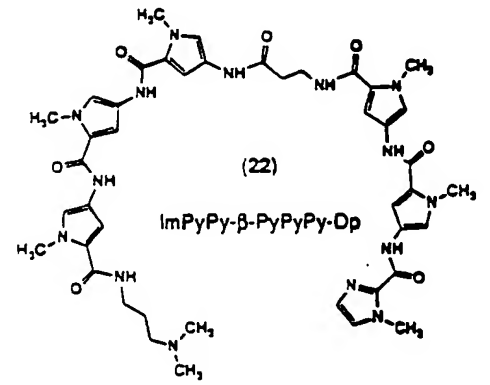
(20)
pyridine-netropsin dimers
n=1-4

3. HAIRPIN POLYAMIDES: A TRIUMPH IN THE DESIGN OF SEQUENCE-SPECIFIC DNA MINOR GROOVE BINDERS

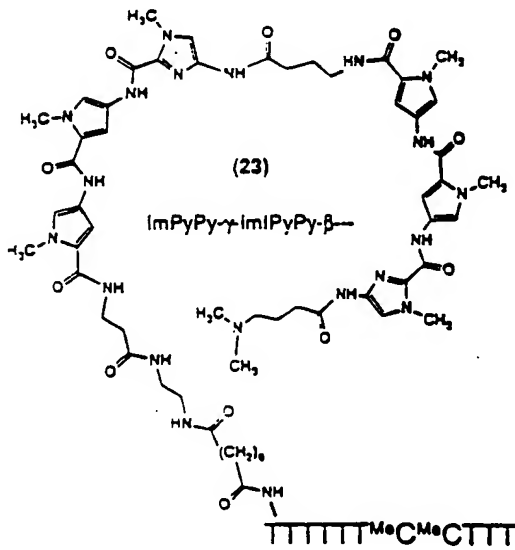
The ambitious goal of rationally designing ligands capable of binding tightly and specifically to any desired target sequence of double-stranded DNA has recently been realized. Hairpin polyamides that consist of two covalently linked lexitropsin molecules connected end-to-end via a γ-aminobutyric acid linker residue, offer promising possibilities for high-affinity specific recognition of a broad sequence repertoire in the minor groove of DNA (*112*–*121*). For examples, hairpin pyrrole–imidazole polyamides **21** and **22** bind specifically to 5′-(A,T)GG(A,T)$_2$ (*112*, *113*) and 5′-AAAAAGACAAAAA (*107*), respectively. Hairpin polyamides can exhibit affinities and specificities for DNA comparable with transcription factors and other DNA binding regulatory proteins (*122*). These molecules, which can be built by solid-phase synthesis (*123*), can discriminate between GC-rich sequences such as GCGC and GGCC, for example (*124*, *125*). The specificity is sufficiently stringent to target a 7 base pair sequence in the minor groove of DNA (*126*). Antiparallel pairing of an imidazole (Im) opposite a pyrrole (Py) residue recognizes a G·C base



(21)
AcPyPyPy-γ-ImImPy-β-Dp



(22)
ImPyPy-β-PyPyPy-Dp



(23)
ImPyPy-γ-ImIPyPy-β—

pair, whereas a Py/Im combination recognizes a C·G pai (Figure 3) (*127*).

Discrimination of T·A versus A·T base pair was a obstacle because a pyrrole/pyrrole (Py/Py) pair is degen erate. The problem has now been resolved. A majo breakthrough has been achieved with the rational desig of new series of hairpin polyamides that can efficientl discriminate T·A from A·T base pairs (*128*). Replacemen of the pyrrole unit with a bulkier 3-hydroxypyrrol enables hairpin polyamide molecules to break the de generacy, allowing the ligand to distinguish A·T and T A pairs (Figure 3). Apparently the increased specificit of hydroxypyrrole(Hp)-containing hairpin polyamide does not arise from enhanced interactions at specific site but mainly comes from enhanced destabilization o interactions at unselected sequences. Placement of H

opposite an adenine residue provokes steric hindrance that destabilizes polyamide binding. In addition, the newly introduced OH group of Hp may engage in hydrogen-bonding interaction with O-2 of a thymine (*128*).

Judicious combinations of the three aromatic amino acids pyrrole (Py), imidazole (Im), and hydroxypyrole (Hp) should permit precise recognition of A·T (Py/Hp), T·A (Hp/Py), G·C (Im/Py), and C·G (Py/im) base pairs (Figure 3) and therefore the targeting of specific sequences. Moreover, the affinity and specificity of hairpin polyamides can be further enhanced via optimization of the γ-turn linker that tethers the two minor groove binding units placed face to face. Replacement of the conventional γ-aminobutyric acid linker with analogues equipped with functional groups (e.g., amino group) can reinforce significantly the affinity as well as the specificity. Stereochemical control of the ligand−DNA interaction has recently been reported using (*R*)- and (*S*)-diaminobutyric acid linker, as depicted in Figure 4 (*129*). Studies of hairpin polyamides are close to success in manipulating gene expression with small molecules. A few problems remain to be solved (e.g., targeting of longer sequences, cellular uptake), but there is no doubt that polyamides are on their way to a promising future as gene-specific control agents (*130*−*134*).

By combining the end-to-end with the side-by-side dimeric motifs, one will perhaps succeed in targeting 15−17 base pairs of unique sequence in a biological system (*135*, *136*). For the first time, it has been shown that an eight-ring hairpin polyamide [ImPyPy-γ-ImPyPyPy-β-Dp] targeted to the binding site of the transcription factor TFIIIA entered into the nucleus of cultured frog fibroblast cells and specifically inhibited transcription of the 5S RNA gene (*137*). Without doubt, with the demonstration of such precise engineering of the hairpin polyamides, a decisive step has been realized toward the targeting of any designated DNA sequence with small molecules.

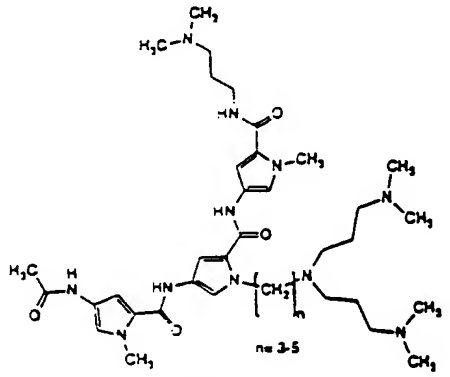
The repertoire of sequences targeted by artificial ligands can be further extended by combining an oligonucleotide with a netropsin-like (*138*) or a hairpin polyamide derivative (*139*). For example, the affinity of the conjugate (23) for the 18 base pair target site 5′-TGACATTAAAAAAGAAA-3′ is 150-fold higher than that of the unlinked subunits (*140*). A related ligand consisting of a pyrimidine oligonucleotide 11 bases in length covalently tethered to an imidazole-containing polyamide cooperatively binds as a dimer to 27 noncontiguous base pairs of double-helical DNA via the formation of a 2:1 ligands/DNA complex (*139*). In both cases, the recognition process involves simultaneous recognition of the minor and major grooves of the double helix, thus mimicking the function of certain sequence-specific DNA binding proteins.

Despite the elegant design strategy for lexitropsin/DNA complexes, the biological activity of minor groove binders has not been much improved. So far, the lexitropsin approach has not led to clinically useful drugs, although certain monomeric and dimeric lexitropsins exhibit interesting antiviral or anticancer activities in vitro and sometimes in vivo (*55*). Some retroamide distamycin analogues exhibit promising antiviral and antiprotozoal activities (*141*).

A considerable number of antitumor agents, including some of clinical value, induce DNA damage either directly by alkylation or cleavage of DNA or indirectly via inhibition of topoisomerase activities. The DNA lesions induced by these different categories of drugs are presumably responsible for their cellular toxicity. Irreversible damage of the genetic material in cells has been considered as the basis of the antitumor effect, but it is more likely responsible, to some extent, for the toxic side effects toward normal cells (genotoxicity). Consequently, there is currently intense interest in the development of sequence-directed DNA damaging agents with a view to improving the therapeutic value of the drugs by virtue of a gene-specific recognition. The following sections describe some examples of netropsin/distamycin conjugates rationally designed to bind tightly to DNA and/or to produce irreparable damages at precisely defined genomic locations.

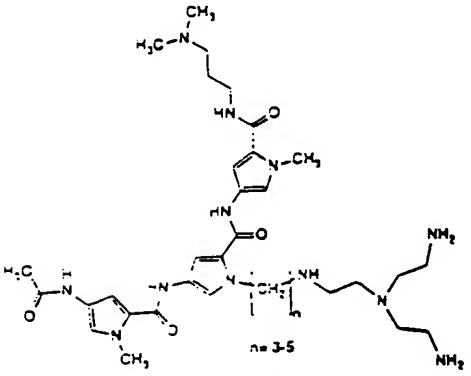
4. LINKAGE TO POLYAMINES

Bruice and co-workers have elaborated a series of molecules they call *microgonotropens* in which the methyl substituent on the central pyrrole ring of distamycin is replaced with a branched polyamine (*142*−*148*). The polyamine substituent on the dien- (24) and tren-microgonotropens (25) projects outward from the minor



(24)

dien-microgonotropen



(25)

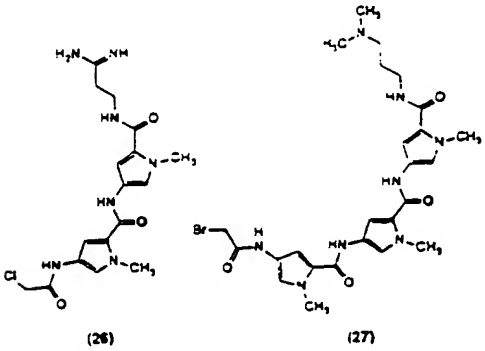
tren-microgonotropen

groove and acts as a hook to increase the affinity for DN via interaction with the phosphodiester backbone or wit the major groove of DNA. The tren [−NHCH$_2$CH$_2$N(CH CH$_2$NH$_2$)] microgonotropen (25, n = 4) is more effectiv than distamycin in promoting DNA bending and straigh ening (*149*). Such compounds can also efficiently compe with the sequence-specific binding of regulatory protein to DNA (*150*). The microgonotropen strategy represen a new avenue in the design of minor groove readir ligands having a very high affinity for DNA. In additio the metal-chelating properties (*151*) of the dien and tre substituents may be exploited for the design of catalys for sequence-selective hydrolysis of DNA, that is, for th design of artificial nucleases (*152*).

5. LINKAGE TO DNA-ALKYLATING AGENTS

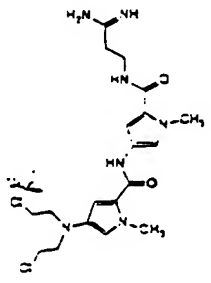
Most alkylating drugs react with DNA via an electrophilic attack on purine residues, in particular on guanines. Classical alkylating agents such as chlorambucil (*153*) and *cis*-platine as well as the pluramycin antibiotics (*154*) react within the major groove of DNA at the N-7 position of guanines. In contrast, mitomycin C (*155*) and anthramycin (*156*, *157*) form adducts with the exocyclic amino group of guanine. Minor groove interactions also occur with the adenine-specific alkylators duocarmycin A and CC-1065 (*158*–*160*).

The accessibility of the major groove and the high intrinsic nucleophilicity of the N-7 heteroatom position contribute to the preferential alkylation of guanine residues by nitrogen mustards. However, the alkylation patterns of nitrogen mustards and nitrosoureas can be modified by linking the reactive group to a DNA reading element. The first generation of alkylating lexitropsins consisted of netropsin and distamycin analogues equipped with *N*-chloroacetyl (**26**) and *N*-bromoacetyl (**27**) substit-



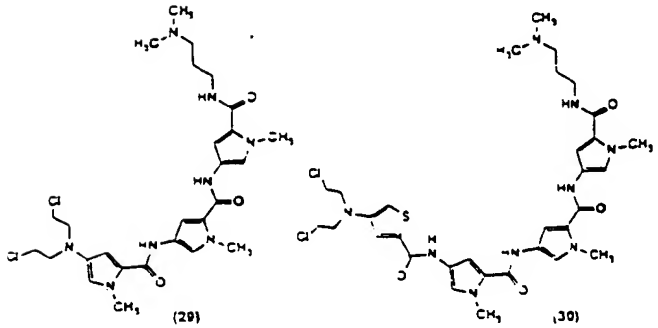
(26) (27)

N-chloroacetyl-netropsin N-bromoacetyl-distamycin



(28)

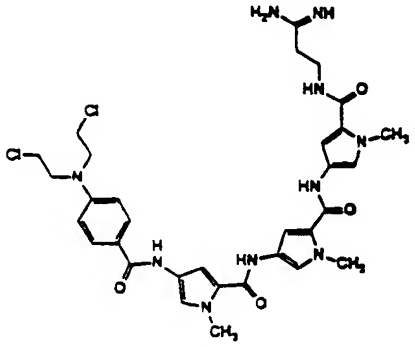
bis(chloroethyl)-netropsin



(29) (30)

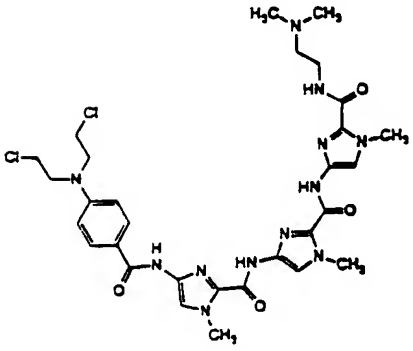
bis(chloroethyl)-distamycins

uents or with bis(chloroethyl)amino substituents (e.g., **28**–**30**) (*161*, *162*). *N*-Bromoacetyldistamycin (**35**) reacts with a single adenine in the sequence 5′-GTTTA·5′-TA*AAC within a 167 base pair restriction fragment (*163*, *164*). The second generation of alkylating lexitropsins contains benzoyl (**31**, **32**) or benzyl mustards (**33**,



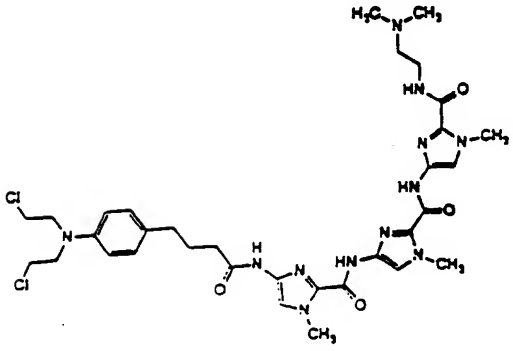
(31)

(N-benzoyl-mustard)-distamycin
FCE 24517



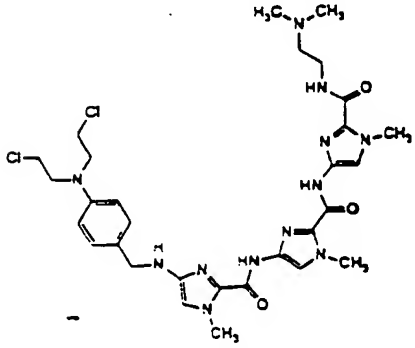
(32)

(N-benzoyl-mustard)-lexitropsin



(33)

chlorambucil-lexitropsin



(34)

(benzyl-mustard)-lexitropsin

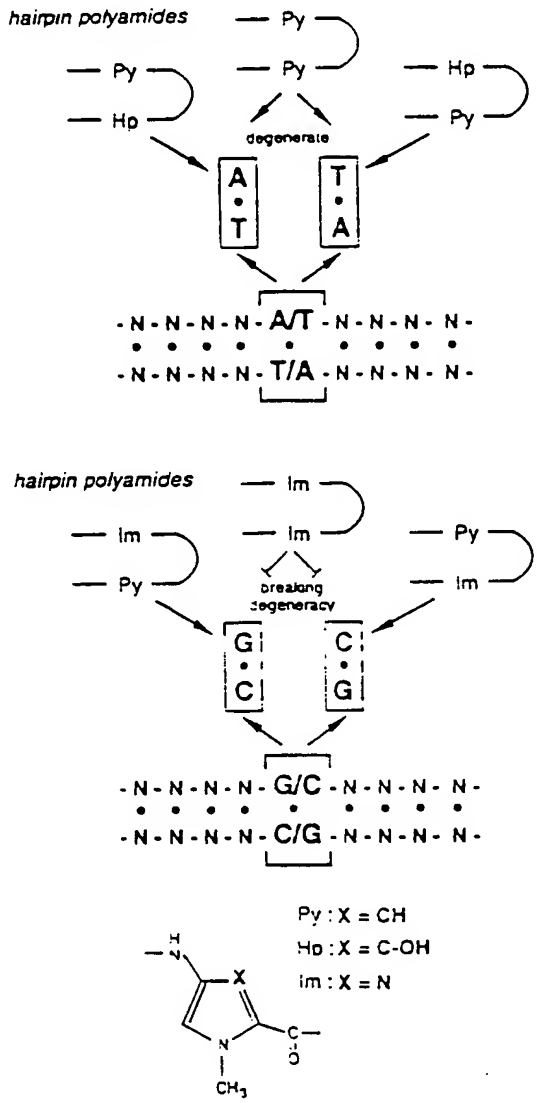
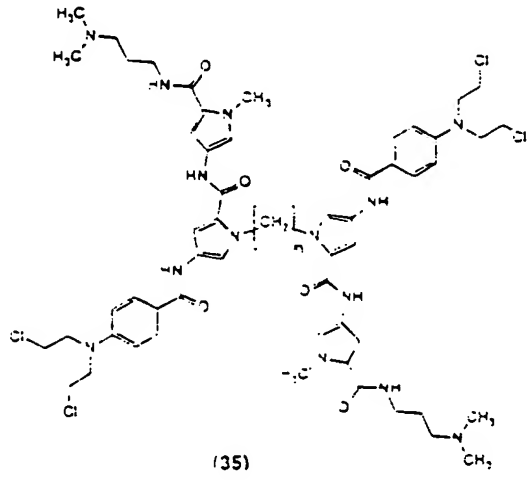


Figure 3. Schematic recognition model of the base pairs by hairpin polyamides containing imidazole (Im), pyrrole (Py), and hydroxypyrrole (Hp) monomers.

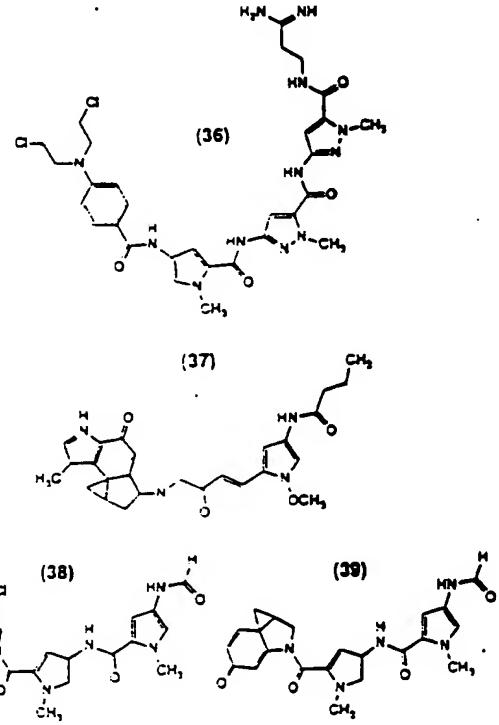
34) (*165–170*). Following the discovery of the aforementioned antiparallel side-by-side motif, bis(mustard) cross-linked lexitropsins such as 35 were synthesized (*171*).



(35)

bismustard-lexitropsin

Distamycin analogues equipped with DNA alkylating functionalities such as compounds **31–34** show a remarkable sequence selectivity with, in some cases, an almost exclusive alkylation of adenines in the minor groove with no detectable guanine N-7 reaction. The lead compound in the series is the bis(2-chloroethyl)aminobenzoyl derivative of distamycin FCE24517 (**31**), also known as tallimustine, which demonstrates significant anticancer activity in animal models and is currently undergoing clinical trials (*172–178*). The antitumor activity of tallimustine is dependent on its capacity to interact with DNA. The drug alkylates the 3′-purine residue within the consensus sequence 5′-TTTTGPu-3′ (*167, 175*). Recently, novel tallimustine derivatives have been synthesized, and some of them, such as the pyrazole derivative **36**, proved to be significantly more active than



tallimustine (*179, 180*). Fifty years after they were introduced into medical practice in the treatment of neoplastic diseases (*181*), nitrogen mustards are still among the most clinically useful anticancer drugs. With the rational design of tumor active drugs such as FCE24517, there is good reason to believe that nitrogen mustards will remain of major clinical importance for a considerable time.

Other series of distamycin conjugates equipped with alkylating functionalities have been synthesized. Lown and collaborators have synthesized a series of lexitropsin cyclopropylpyrroloindole (CPI) hybrids (*182*). The CPI–*N*-methylpyrrolecarboxamide derivative (**37**) exhibits an exceptional cytotoxic potency against KB tumor cells in vitro (IC_{50} = 0.76 fg/L) and forms stable covalent adducts in the minor groove of DNA (*183*). The pharmacophore found in CC-1065 and the duocarmycins has been exploited for the design of conjugates **38** and **39**. Compound **38**, containing a stable 3-(chloromethyl)-6-hydroxyl-2,3-dihydroindole, binds covalently to AT-rich sequences in DNA (*184*). Church et al. (*185*) have synthesized chloroethylnitrosourea–lexitropsin conjugates such as **40**, which alkylates adenine residues in the minor groove. Zhang et al. (*90*) have prepared a series of noncationic *N*-methylpyrrole dipeptides incorporating sulfonate ester terminal groups (**41**, **42**). Here also, efficient alkylation

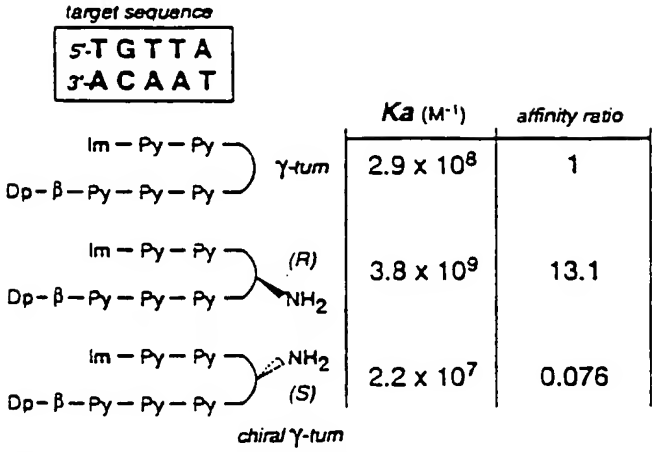
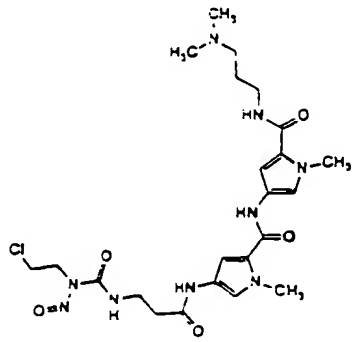
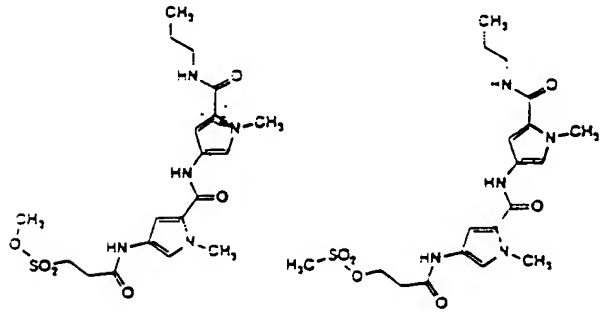


Figure 4. Influence of the linker. Replacement of the γ-aminobutyric acid linker of the hairpin polyamide ImPyPy-γ-PyPyPy-β-Dp targeting the sequence 5'-TGTTA with a chiral 2,4-diaminobutyric acid linker changes considerably the equilibrium binding constants K_a, increasing (R) or decreasing (S) binding affinity relative to the nonchiral γ-turn linker.



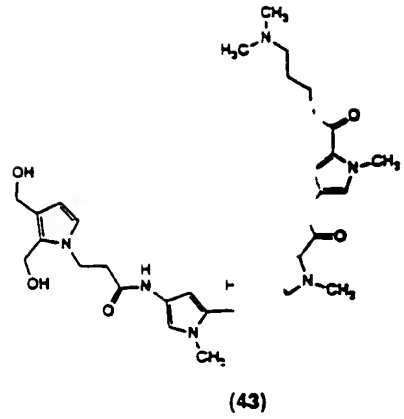
(40)

Cl-ENU-netropsin



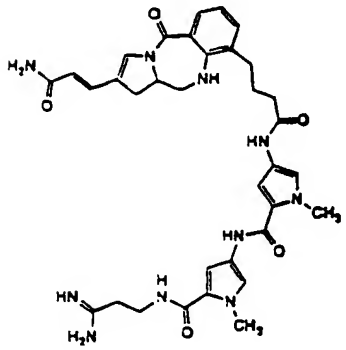
(41) (42)

methylsulfonate-netropsins



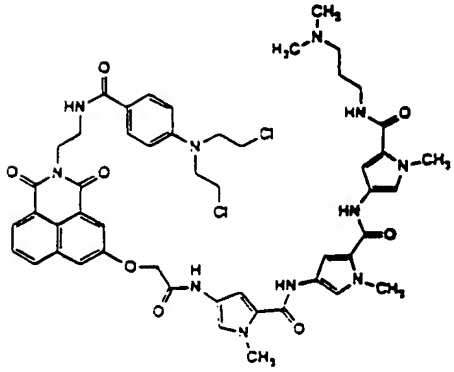
(43)

[bis-(hydroxymethyl)-pyrrole]-distamycin



(44)

anthramycin-netropsin

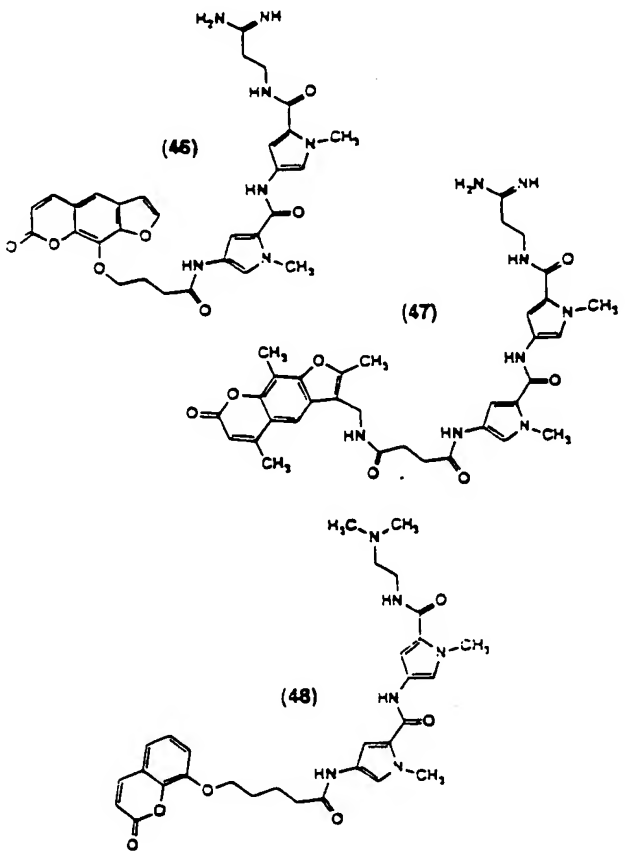


(45)

at adenine N-3 in the minor groove was observed. An elegant way to provoke DNA–DNA cross-links has been reported using a distamycin analogue coupled to a 2,3-bis(hydroxymethyl)pyrrole function, which in part mimics the functionality present in reductively activated mitomycins or oxidatively activated pyrrolizidine alkaloids. Compound 43 efficiently produces interstrand cross-links by bridging the 2-amino groups of two paired CpG steps (*186*, *137*). More recently, Walker et al. (*188*) have designed netropsin–anthramycin conjugates such as the chimera 44, which is expected to recognize the sequence RGAAAA from the HIV-1 polypurine tract. Gupta et al. recently reported the synthesis, DNA binding, sequence specificity, and biological evaluation of DNA-directed alkylating agents comprising naphthalimide, nitrogen mustard, and lexitropsin moieties (e.g., compound 45). However, in this case alkylation still occurred at N-7 guanine, indicating that the bulky intercalative naphthalimide mustard does not enhance sequence specificity (*189*).

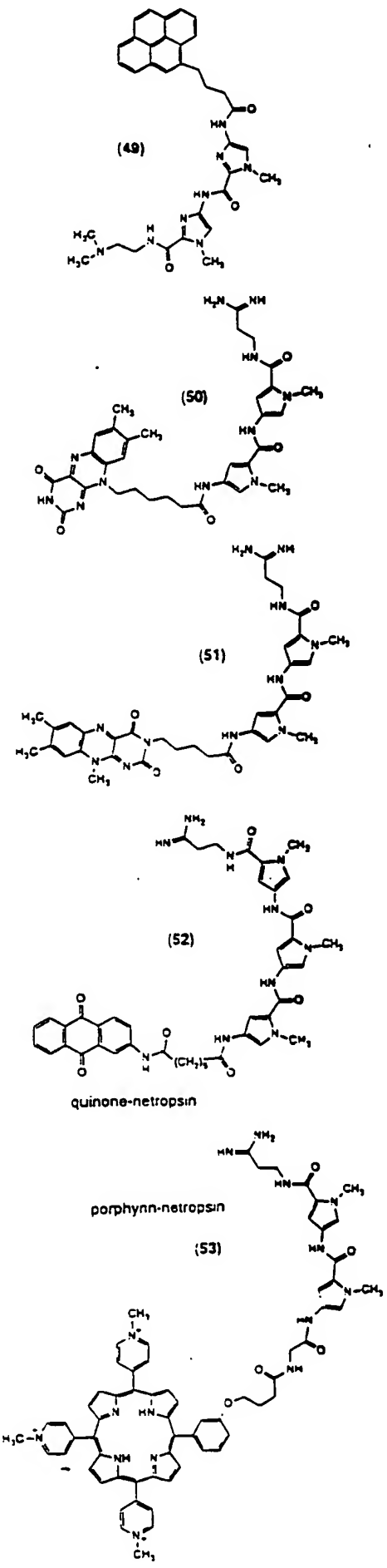
6. LINKAGE TO A PHOTOSENSITIVE GROUP

A few psoralen derivatives (e.g., 8-methoxypsoralen) are used in phototherapy for the treatment of human skin diseases, chronic leukemia, and some infections connected with AIDS. The biological effect probably arises from intercalation into DNA. When exposed to UV light, intercalated molecules react covalently with DNA to form cyclobutane links to pyrimidine bases, predominantly at the 5'-TpA step (*190*). Psoralen (46, 47) and coumarin (48) derivatized minor groove binding oligopeptides have been used to induce light-dependent sequence-specific reaction with DNA (*191*, *192*). Pyrene–lexitropsin con-

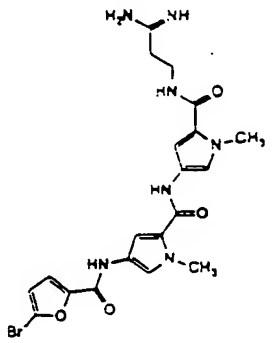


jugates such as (49) were also synthesized (*193*). The cytotoxicity of this sequence-selective photosensitizer is significantly enhanced upon irradiation. The photoinduced DNA lesions apparently result from the production of singlet oxygen. Using a similar approach, Herfeld et al. (*194*) have synthesized netropsin–isoalloxazine conjugates such as compounds **50** and **51**. Upon photoactivation in the presence of molecular oxygen, the flavin chromophore oxidizes and generates oxy radicals capable of causing DNA breaks. The linkage of netropsin to a flavin chromophore leads to AT selective strand cleavage reaction (*195*, *196*). Quinone–netropsin hybrids (e.g., **52**) have been also designed. These conjugates are capable of inducing UV-mediated strand breaks (*197*).

Cationic porphyrins can also act as DNA photosensitizers (*198*). Molecular modeling predicts that the porphyrin moiety of the conjugate **53** intercalates into DNA (*199*, *200*). UV-sensitive *p*-nitrobenzoyl groups attached to netropsin–acridine hybrids also act as photocleavers. Matsumoto et al. (*201*) have synthesized a series of oligo(*N*-methylpyrrolecarboxamide) derivatives linked to halogenated heteroaromatic groups. Photoinduced DNA cleavage by the bromofuran–netropsin conjugate **54** is not mediated via active oxygen species ($OH^{\bullet}$) but may be due to the reaction of an aryl radical produced upon photohomolysis of the carbon–halogen bond (*201*). A similar mechanism with drug radical production has been proposed for X-ray-sensitive nitroaromatic compounds. Metronidazole and misonidazole are typical examples of radiosensitizing agents used in the treatment of anaerobic infections and are under continuing investigation regarding their use in cancer therapy, acting as markers for hypoxic regions in tumors (*202*). These two sensitizers only react only weakly with DNA, presumably via an electron-seeking radical (*203*, *204*). Targeting of a nitroarene moiety to DNA via a minor groove binder is expected to increase the sensitizer concentrations at

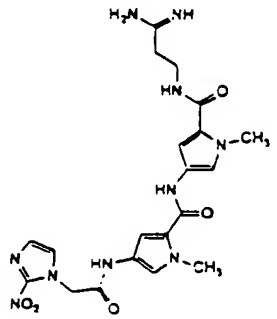


defined sites on DNA (*205*–*207*). The affinity of the 2-nitroimidazole–netropsin conjugate (55) for DNA is



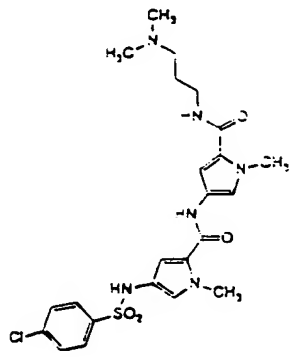
(54)

bromofuran-netropsin



(55)

misonidazole-netropsin

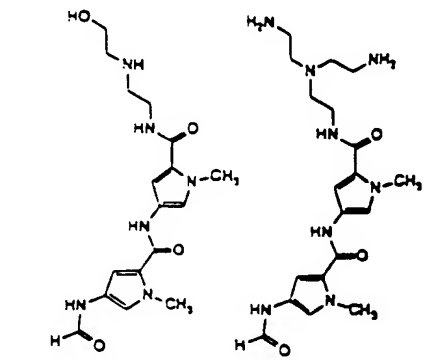


(56)

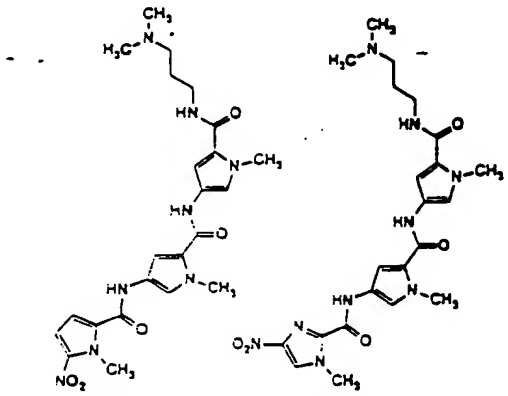
p-chlorobenzylsulfonamido-netropsin

~200-fold superior to that of misonidazole, but despite the improved interaction with DNA and improved cellular uptake capacity, the radiosenzitization efficiency remains relativeiy poor and not better than that of misonidazole (*206*). Photoinduced DNA cleavage has been reported with oligo-*N*-methylpyrrolecarboxamide derivatives substituted with a benzylsulfonamido group such as the *p*-chlorobenzenesulfonamide–netropsin hybrid **56**. In this case, the efficiency of single-strand cleavage under UV-A irradiation clearly depends on the length of the pyrrolecarboxamide chain. Tetrapyrrole–sulfonamide conjugates are more efficient DNA cleavers than the corresponding analogues containing three or two pyrrole units, and conjugates with only one pyrrole are practically inactive (*208*). The same conclusion was drawn for simple oligopeptides such as **57** and **58**, which do not possess special side chains sensitive to UV light but which, nevertheless, can induce DNA cleavage under UV-A irradiation (*209*). Conversely, for nitrated oligopyrrolecarboxamide derivatives such as **59** and **60**, the DNA photocleavage efficiency is higher for the monopyrrole compounds than for the bis- and tris(peptide)s (*210*).

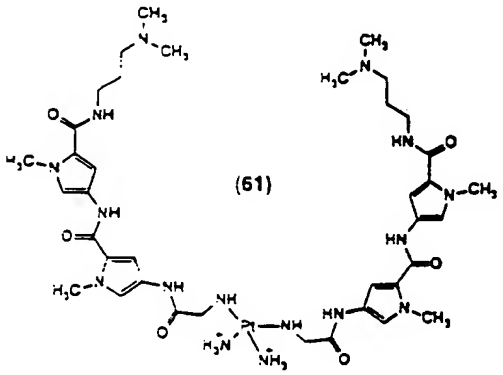
The netropsin–platinum–netropsin conjugate (**61**) represents another type of netropsin conjugate capable of inducing DNA cleavage upon X-ray ionization (*211*). According to footprinting experiments, the drug binds selectively to 9 base pair long AT-rich sites with the two netropsin moieties extended along the minor groove. Recently it was shown that along with the extended conformation, hairpin conformation can coexist with the pyrrole rings of one netropsin-like element stacked on the pyrrole rings of the other. This structure is reminiscent of the parallel hairpin polyamide motif described above (*212*). X-irradiation of drug/DNA complexes yields discrete cutting sites near the center of the platinum–bis(netropsin) binding sites. The cleavage would result from the rupture of the deoxyribose residues upon attack by Auger electrons generated by the irradiated platinum atom. Such conjugate compounds capable of triggering sequence-specific DNA degradation might be of interest for X-ray therapy of tumors.



(57) (58)



(59) (60)



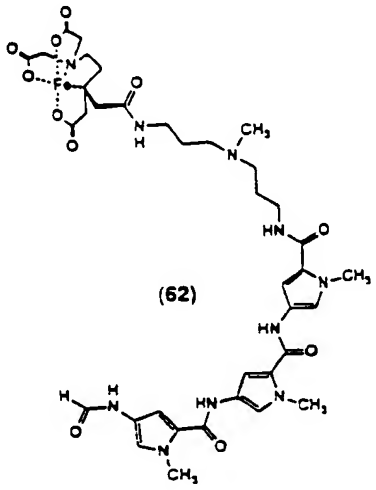
(61)

netropsin-platinum-netropsin

7. LINKAGE TO A METAL COMPLEX

Endonucleases, such as DNase I, are generally employed to map ligand binding sites on DNA (*213*, *214*), but the preequilibrated ligand/DNA complex can be subjected to the nicking activity of a chemical nuclease, such as Dervan's reagent methidiumpropyl-EDTA complexed with iron (MPE·Fe^{II}). It is also possible to equip the ligand with its own DNA-cleaving functionality, for example, to attach an Fe·EDTA complex to the test drug. This method, termed DNA-affinity cleaving, has proved

...be very successful in analyzing binding sites for distamycin via the use of EDTA–distamycin conjugates such as compound 62 (*214–216*). The technique has been

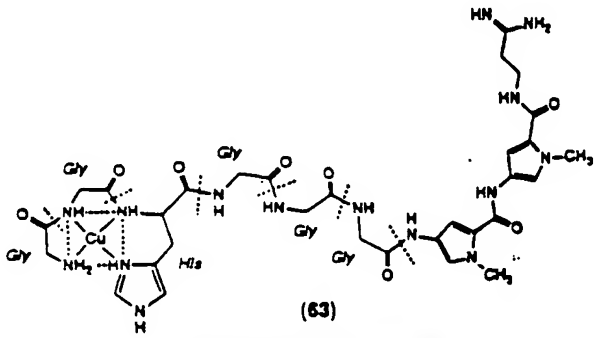


distamycin-EDTA

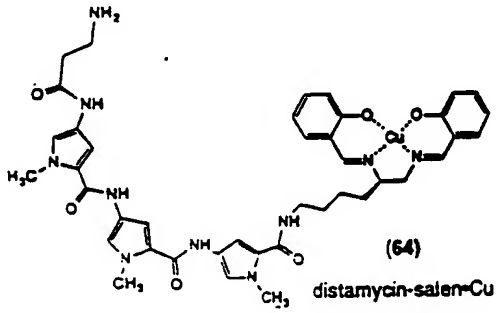
extensively exploited to study oligo(*N*-methylpyrrolecarboxamide) derivatives containing up to 12 pyrrole units as well as a variety of monomer and dimer lexitropsins (*217–222*). Upon chemical activation with a reducing agent (e.g., dithiothreitol) the iron–EDTA portion generates hydroxyl radicals, which react with the deoxyribose residues to produce DNA strand cleavage. In contrast to Fe·EDTA footprinting, the active oxygen species are produced directly in the vicinity of the ligand binding sites on DNA and so are less susceptible to diffusion.

Metal complexing peptides can serve as a source of oxygen radicals. The growth-modulating tripeptide glycyl–histidyl–lysyl (GHK) and the related tripeptide glycyl–glycyl–L-histidine (GGH) both form complexes with copper and upon activation can generate oxygen active species (*223–226*). Linkage of the metal-complexed GHK peptide to minor groove binding drugs has been considered as a means of inducing sequence selective cleavage. Compound **63** was synthesized. The peptide moiety not only cleaves DNA but also contributes positively to the DNA binding reaction (*227, 228*). Nonpeptidic metal complexes have been utilized. Recently, Dst was linked to a bis(salicylidene)ethylenediamine derivative to give the distamycin–salen conjugate **64**. However, the cleavage remained largely nonspecific (*229*).

The best characterized natural model for sequence-specific DNA cleavage is bleomycin, which remains one of the most useful antitumor drugs. The bleomycin·Fe(II) complex combines with O_2 to produce a reactive oxygenated metallobleomycin species, which is capable of abstracting a hydrogen atom from the C4′ position of deoxyriboses in DNA. Bleomycin generates mainly single-strand breaks at pyrimidine residues 3′ to a guanine residue (i.e., at 5′-GpC and 5′-GpT sequences) (*230*). The long established clinical utility of bleomycin sparked tremendous interest in its mechanism of action and in the design of compounds based on its structure. A considerable number of bleomycin analogues and related structures have been designed, including some equipped with DNA reading elements based on netropsin and distamycin. The structure of the antibiotic has been simplified to yield analogues such as PYML, PMAH (*231*), and AMPHIS (*232–234*), which mimic efficiently the metal-chelating/oxygen activation domain of bleomycin.



Gly-Gly-His -(Gly)$_3$-netropsin



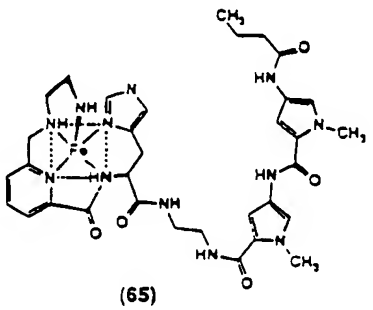
distamycin-salen-Cu

Attachment of PYML (*235, 236*) and AMPHIS (*237–241*) to lexitropsin carriers gave bleomycin-like conjugates such as compounds **65–68** endowed with interesting sequence-specific DNA recognition properties. For bleomycin, the whole molecule spanning from the pyrimidine region to the bithiazole terminus appears to be responsible for specific recognition of DNA (*242–244*). In contrast, the lexitropsin moiety is only responsible for specific base recognition of man-designed bleomycin conjugates. The fact that the synthetic PYML–distamycin hybrid **67** is more toxic than bleomycin itself toward L1210 leukemia cells in vitro encourages the design of other related bleomycin-like molecules tailored with DNA reading elements.

8. LINKAGE TO AN ENEDIYNE LIGAND

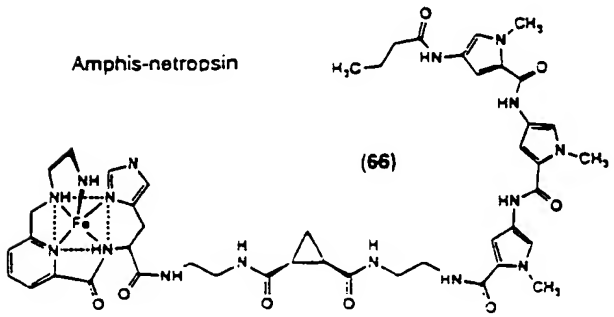
The discoveries in the late 1980s of the antitumor activity of enediyne antibiotics rapidly elicited extensive research activity into their chemistry. An impressive number of synthetic analogues of dynemicin, neocarzinostatin, calichaemicin, and esperamicin have been described (*245–247*). Upon activation, enediynes trigger a Bergman reaction, which leads to highly reactive benzenoid diradicals and causes severe DNA damage(*247*). Enediyne antibiotics are among the most cytotoxic compounds known so far, and their activity is likely attributable to their effect on DNA. The excessive reactivity of the enediyne moiety prompted the development of analogues containing DNA delivery systems. To this end, netropsin was coupled with the neocarzinostatin chromophore (**69**) (*248*) and distamycin was attached to a dynemicin model (**70**) (*245*). More recently, the synthesis and biological activity of new netropsin-enediyne hybrids such as **71** was reported. The addition of the binding domain improved the DNA cleavage potency considerably (up to 100-fold), but a concomitant increase in potency against tumor cells was not observed (*249*).

Simple cyclic enediynes attached to netropsin moieties have also been elaborated (*250*). The linkage of the two functionalities via acetate and crotonate (**72**) tethers results in a hybrid series in which the cleavage efficiency is strongly enhanced (up to 1000-fold compared to the

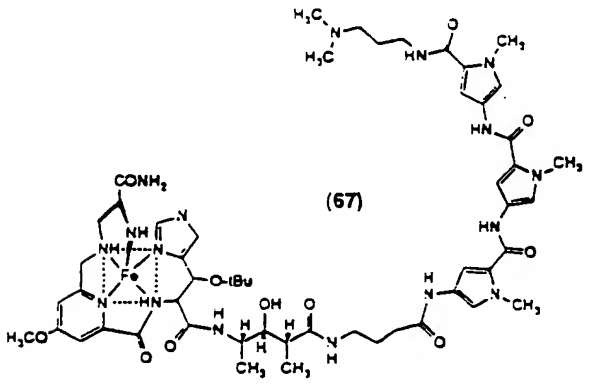


(65)

Amphis-netropsin

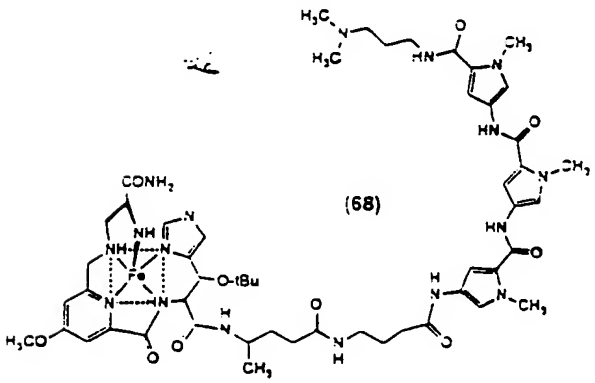


(66)



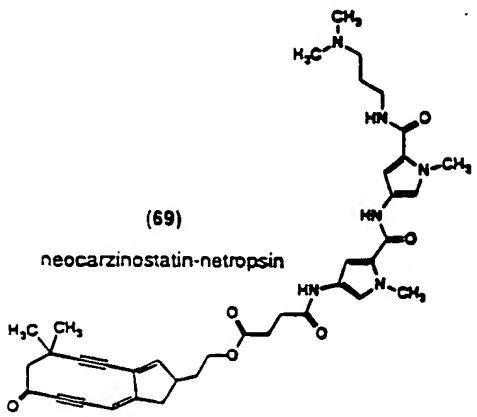
(67)

PYML(6)-(AHM)-distamycin



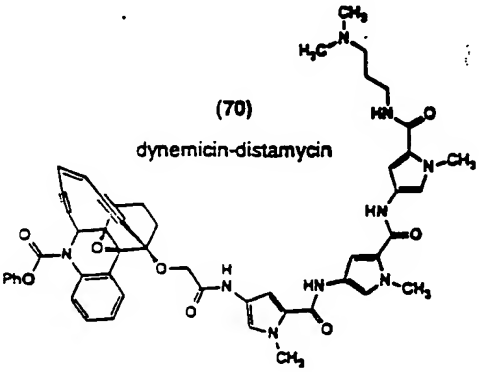
(68)

PYML(6)-(APA)-distamycin



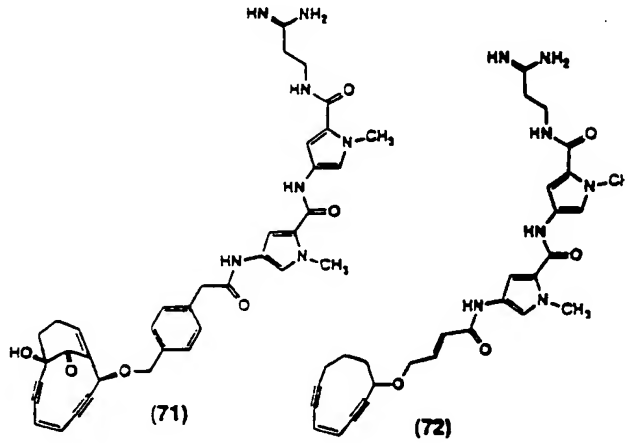
(69)

neocarzinostatin-netropsin



(70)

dynemicin-distamycin



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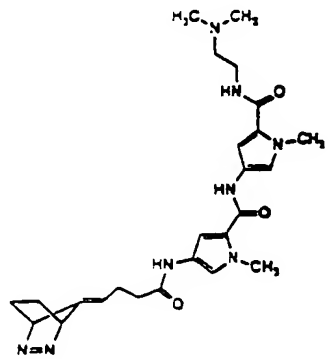
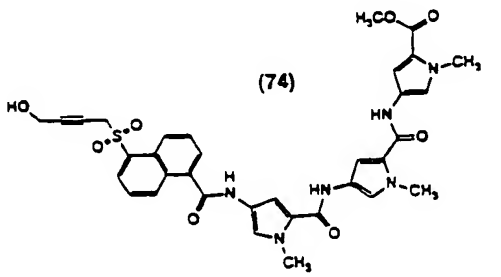


(73)

TMM-netropsin

enediyne alone) (*251*). These encouraging results pave the way for the design of simpler related structures capable of triggering effective DNA cleavage via a radical mechanism. In this context, Bregant et al. (*252*) synthesized the netropsin analogue 73 equipped with a trimethylenemethane group (TMM), which can undergo cycloaddition to electron deficient alkenes. Upon photolysis of the diazene moiety, the TMM−netropsin conjugate can transform to a diyl radical and cleaves DNA, predominantly at AT-rich regions (*253*). Propargylic sulfones are small synthetic molecules that mimic the chemical action of enediynes. They can cleave DNA in pH-dependent fashion. Here again, linkage of lexitropsi carriers to propargylic sulfones (74, 75) may permit th cleavage to be directed to specific sequences in DN (*254*). It is worth mentioning here the netropsin



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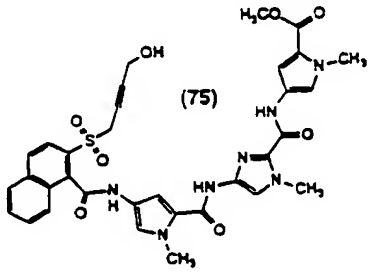
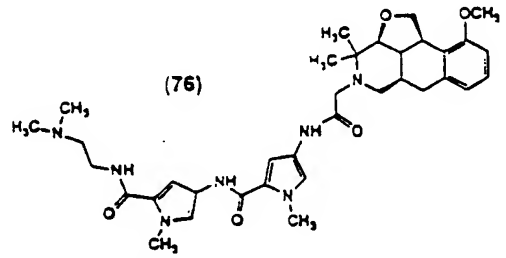


(75)



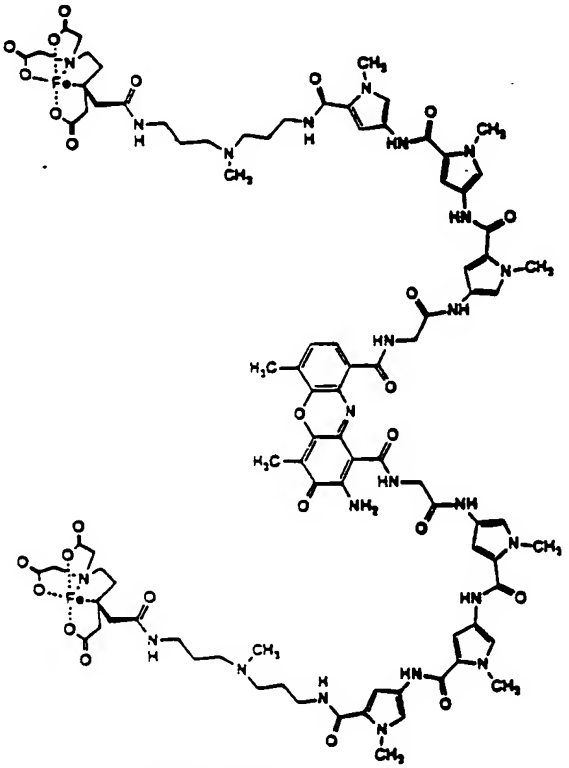
(76)

quinocarcin conjugate **76**, which can efficiently cleave DNA at AT-rich sequences via the production of a nondiffusible oxidant (*255*).

9. LINKAGE TO AN INTERCALATING AGENT: THE COMBILEXINS

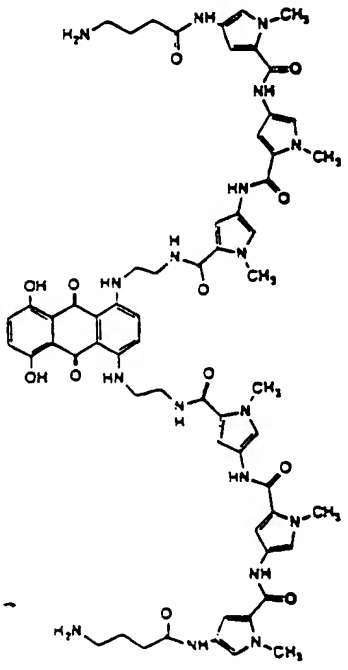
A large number of intercalating drugs, including some of major clinical value, possess a heterocyclic chromophore substituted with peptide, alkyl, or carbohydrate side chains that participate in DNA recognition. These chains represent a kind of hook for sequence-selective interaction within the minor groove of DNA. For example, the antitumor drug actinomycin intercalates between base pairs, leaving the two pentapeptide lactones lying in the minor groove of the double helix (*256–258*). The symmetrically disposed cyclic peptides participate in the specific recognition of GpC sites by actinomycin. Similar observations can be made for a variety of tumor active intercalating drugs. Anthracyclines such as daunomycin and nogalamycin contain carbohydrate residues that serve as DNA recognition elements (*259, 260*). The peptide rings of the quinoxaline antibiotics echinomycin and triostin A are prime determinants for sequence-specific recognition by the drug (*261*). The enediyne antibiotics dynemicin A (*262*) and neocarzinostatin (*263*) bind to DNA by intercalation of their chromophore (naphthoate for neocarzinostatin and anthraquinone for dynemicin), placing their reactive enediyne-containing bicyclic core moiety in the minor groove in a suitable position for selective DNA cleavage. Such considerations indicate that in many cases drugs usually referred to as "intercalators" in fact exhibit mixed modes of binding to DNA and therefore should be considered as intercalator–minor groove reading hybrid molecules or naturally occurring *combilexins* (thus called by analogy with the *iexitropsins*) (*264, 265*).

Using actinomycin D as a model compound, Krivtsova et al. (*266*) designed a series of hybrid molecules called distactins in which the phenoxazone chromophore is substituted at positions 1 and 9 with one, two, or three *N*-methylpyrrolecarboxamide units. Bidentate reaction with DNA involving intercalation of the chromophore and minor groove binding of the DNA reading element was observed with distactins bearing one or two pyrrole rings but not with the analogues having three units. The model was reconsidered two years later (*45*). DNA affinity cleaving studies of the bis(EDTA–distamycin)–phenoxazone conjugate molecule **77** revealed a major



distactin (77)

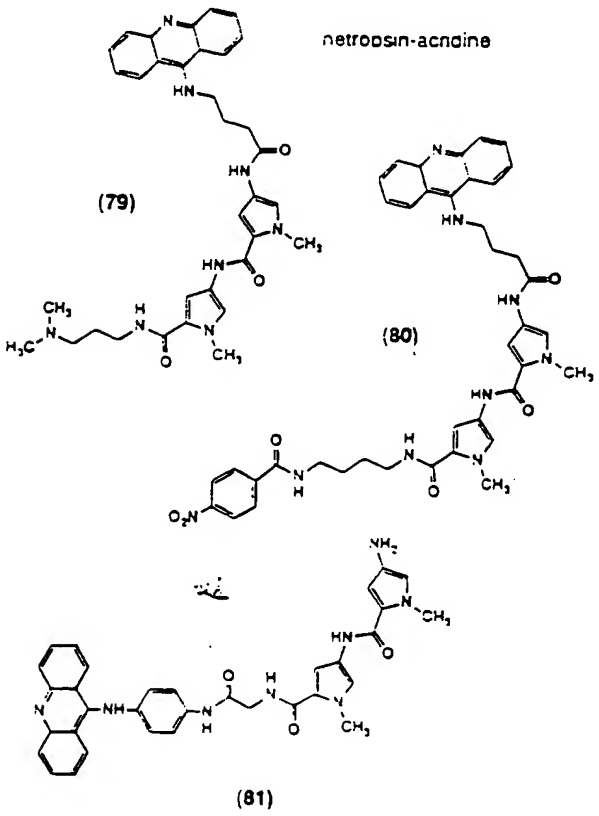
[bis-(EDTA-distamycin)]-phenoxazone



NetMitox (78)

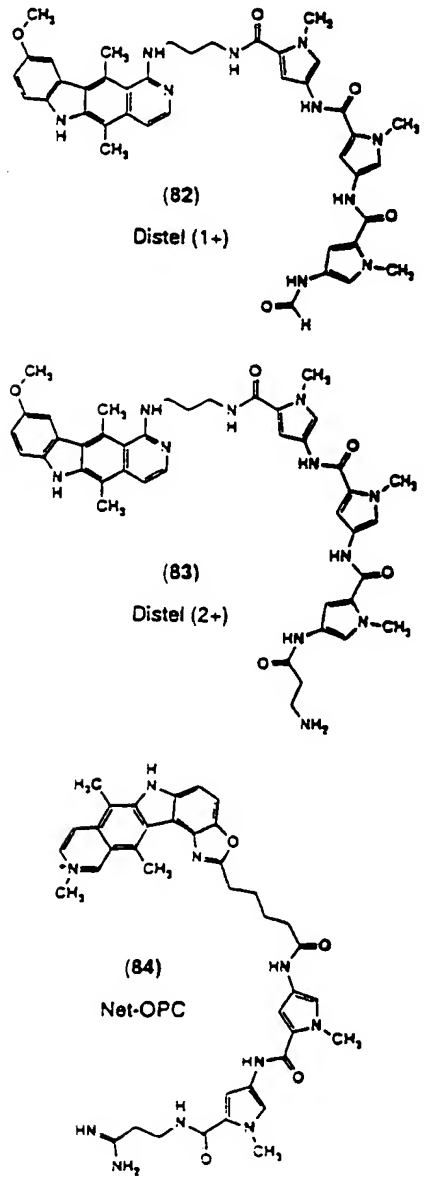
cleavage site flanking the 10 base pair sequence 5′-TATAGGTTAA, suggesting thus that intercalation of the tricyclic nucleus at the central GG step is accompanied with minor groove binding of the distamycin moieties at the flanking $(A\text{-}T)_4$ sites. Additional single cleavage loci were also observed. Depending on the recognized sequence, only one or both recognition elements engage in contact with DNA (*45*). Similar results were recently obtained with the bis(netropsin)−anthraquinone combilexin 78. Intercalation of the mitoxantrone-derived chromophore is hindered by the appended minor groove elements. The anthraquinone not only provides extra strength of binding but notably influences the DNA recognition by the minor groove element (*267*).

Bis(pyrrolecarboxamide) moieties were linked to a 9-aminoacridine chromophore by alkyl linkers of variable length (*268*). Optimum fit to DNA was obtained with the combilexin 79 with a butyroyl tether. This biscationic hybrid exhibits a strict AT preference as for distamycin. A structurally related acridine−distamycin ligand equipped with a photoactivatable *p*-nitrobenzoyl (80)



group has been synthesized (*269*). This conjugate can cut DNA upon activation with UV light (310 nm) with a preference for AT sequences (*270*). The selectivity for AT sites was found to be significantly decreased when a truncated netropsin moiety was linked to a glycylanilinoacridine chromophore structurally related to the antileukemic drug amsacrine. In this case, the rigid connector between the bis(pyrrole) unit and the acridine nucleus of the combilexin molecule 81 does not permit optimal intercalation of the acridine and apparently slightly restricts the netropsin moiety fitting deeply into the minor groove (*271*, *272*). However, this compound is a potent topoisomerase II inhibitor and exhibits moderate antitumor activities in vivo (*273*, *274*). Two functional domains were identified in the combilexin 81: the anilino group can be regarded as a skeletal core to which are connected, on the one side, the tricyclic acridine moie which represents the DNA binding domain, and, on t other side, the *N*-methylpyrrolecarboxamide moie which constitutes the topoisomerase II-targeted doma (*275*).

Covalent linkage of distamycin to a GC-selective · lipticine derivative (*276*) afforded a monocationic hybr molecule Distel(1+) (82), capable of bidentate binding DNA. The reaction with DNA was primarily driven : the charged ellipticine moiety of the hybrid (*277*−*27* The ellipticine chromophore has markedly reinforced t affinity of the ligand for DNA, but the effect is at tl expense of DNA sequence selectivity. Computation studies suggested that the addition of a positively charg group on the distamycin terminal group would fav binding to AT sequences (*277*). The calculation prove to be correct. Indeed, the substitution of the termin formamido group of Distel(1+) for an aminopropionamic group charged at neutral pH [Distel(2+) (83)] was tl



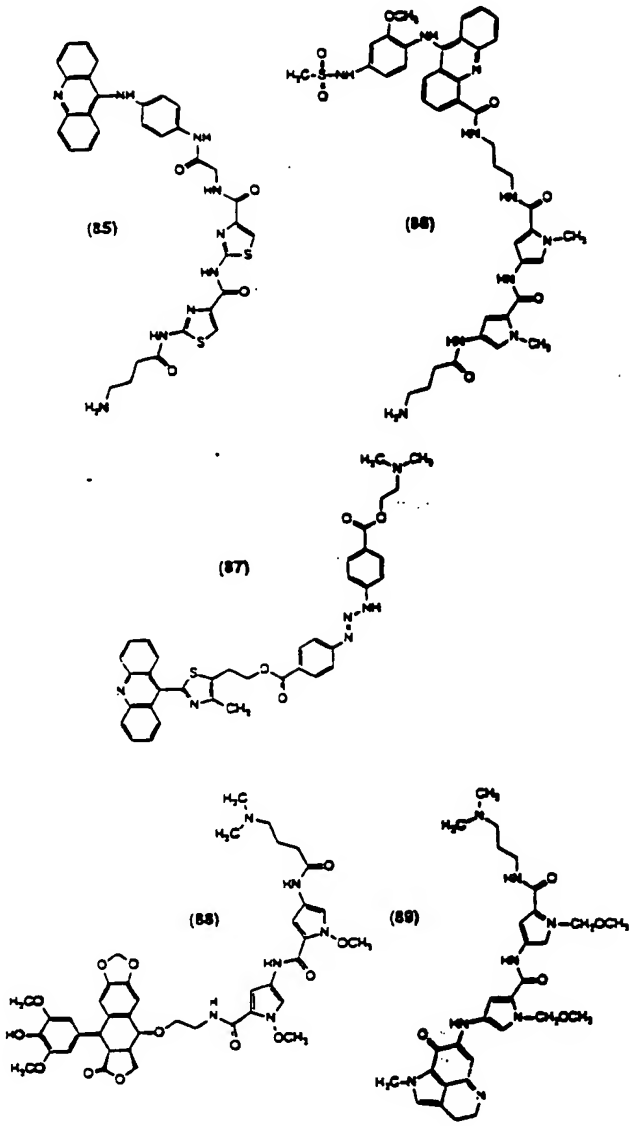
correct way to proceed to convert a nonspecific conjugate into a highly AT-specific DNA reader (*280*). In contrast to Distel(1+), the interaction of Distel(2+) with DNA seems to be driven as much by the distamycin moiety as by the ellipticine residue. Of the two distamycin−

...ticine hybrids 82 and 83, only the monocationic ...brid Distel(1+) (82) proved to be a topoisomerase ...hibitor. Its biscationic analogue Distel(2+) (83) showed practically no effect on both topoisomerase I and topoisomerase II, despite its superior DNA binding properties (*231*). The poisoning of topoisomerase I by Distel(1+) contributes to the cytotoxic effect since P388CPT5 cells resistant to camptothecin (a powerful topoisomerase I inhibitor) display a notable cross-resistance to Distel(1+) (*231*).

A different situation has been reported with a biscationic netropsin conjugate containing an oxazolopyridocarbazole chromophore derived from the ellipticines. The Net-OPC (84) hybrid ligand adopts different configurations according to the sequence to which it binds. At GC sites, the OPC chromophore intercalates into DNA but the netropsin remains unbound. At AT sites, the most energetically favored complex has both the netropsin and the OPC moieties inserted in the minor groove of a 7 base pair long sequence. In contrast, the second favored complex at $(A\cdot T)_4$ sites involved intercalation of the OPC ring and minor groove recognition by the bispyrrole moiety (*282–285*). A somewhat similar coexistence of an intercalative and a nonintercalative binding mode was reported for the netropsin–porphyrin conjugate 53 (*199*). The connecting group between the two DNA binding elements plays a critical role in the DNA recognition process (*200*). These typical examples illustrate the difficulties encountered in designing combilexin molecules. It is a challenging exercise that demands consideration of the notions of geometrical compatibility, hydrogen bonding capability, and the overall electronic properties of the interacting species.

A moderate antitumor activity in vivo was observed with the bifunctional molecule 85, which combines features of both the lexitropsin and combilexin approaches. This hybrid ligand, in which are conjugated the thiazole lexitropsin (12) and the intercalating anilinoacridine chromophore, binds to DNA via a bimodal process involving minor groove binding of the lexitropsin moiety and intercalation of the acridine moiety (*286*).

A second generation of combilexins has now been designed. Unlike compound 81, the netropsin–amsacrine hybrid (86) bears a positively charged terminal side chain, which contributes significantly to the ATT selectivity of such ligands and retains the *m*-methoxy and methanesulfonamide substituents on the anilino ring, which constitute key elements for the interference with topoisomerases, the maintenance of redox properties, and the biological properties of amsacrine. The netropsin moiety is connected to the acridine ring via a 4-carboxamide side chain, whereas it was previously attached directly to the anilino group. Linkage of a carboxamide side chain to position 4 of the acridine ring of amsacrine has earlier been shown to convert the drug from a classical intercalator to a threading intercalator (*287, 288*). Structural and kinetic studies have revealed that the conjugate threads through the DNA double helix so as to intercalate its acridine chromophore, leaving the netropsin moiety and the methanesulfonanilino group positioned within the minor and major grooves of the double helix, respectively (*259*). In addition, the hybrid retains the susceptibility to copper-dependent oxidation and to generate DNA-damaging oxygen radicals (*290*). It also stimulates the formation of cleavable complexes with DNA in the presence of topoisomerase II, but its netropsin-like moiety confers little or no influence on the reaction with the topoisomerase (*290*).



Thus far, the combilexin strategy has concerned netropsin- or lexitropsin-like minor groove binders attached to different intercalating chromophores. In 1995, the strategy was extended to another category of minor groove binders, namely, 1,3-diaryltriazenes derived from the antiviral and antiprotozoal drug berenil. The acridine–triazene combilexin (87) exhibits a distinct preference for AT base tracts rather than GC-rich sequences and is ~40-fold more cytotoxic than the triazene or acridine subunits toward L1210 mouse leukemia and A2780 human colon cancer cell lines (*291*). These results on combilexin molecules obtained so far encourage us to believe that this approach to DNA-targeted pharmacology has the potential to yield important developments in the search for new classes of topoisomerase inhibitors and perhaps for new and better anticancer drugs.

The epipodophyllotoxins etoposide (VP-16-213) and teniposide (VM-26) are very potent topoisomerase II inhibitors and are mainly used in chemotherapy for the treatment of small cell lung cancer, testicular cancer, lymphoma, and leukemia. These compounds stabilize DNA/topoisomerase II complexes but interact only weakly if at all, with DNA in the absence of the enzyme. Lown and co-workers have attempted to direct epipodophyllotoxins to specific sequences via the conjugation with lexitropsins. A series of 4′-demethylepipodophyllotoxin-lexitropsin conjugates such as compound 88 were syn

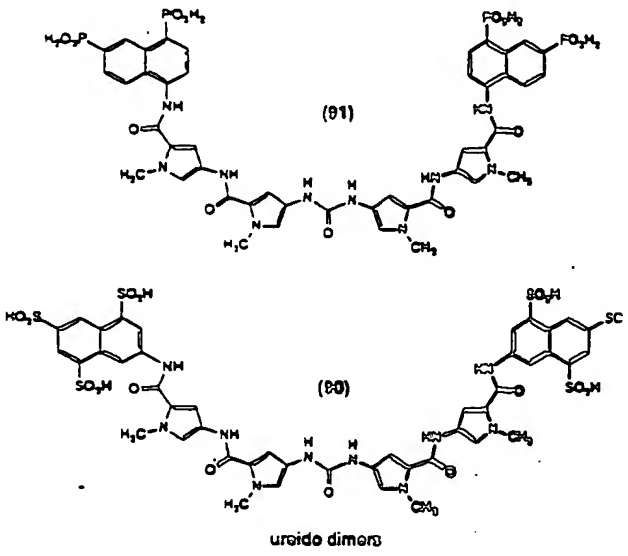
thesized, but these compounds were found to be much less cytotoxic than the parent compounds (*292*). In the same vein, a pyrroloquinoline nucleus of the topoisomerase II inhibitor makaluvamine A has been linked to mono-, bis-, and tris(pyrrolecarboxamide) moieties. Hybrids such as compound 89 show significant cytotoxicity against different tumor cell lines (*293*).

10. CONCLUSION

Netropsin and distamycin have greatly contributed to our understanding of the molecular basis of drug–DNA recognition. The understanding of how these two antibiotics recognize and bind to AT sequences in DNA provided perceptions of how to design sequence-specific DNA binding molecules and stimulated the synthesis of several classes of new compounds of potential use in cancer chemotherapy. As shown in this review, the chemical structure of netropsin-like DNA reading elements may be varied in many synthetically convenient fashions to produce different repertoires of sequence-selective DNA binding molecules with a range of functionalities. Sterically demanding groups can be attached to netropsin and distamycin without abolishing their sequence recognition properties. The fact that certain netropsin analogues can readily recognize a specific sequence in DNA (e.g., lexitropsins forming 2:1 complexes and hairpin polyamides) shows that DNA targeting with minor groove binding drugs is no longer merely a possibility but a practical reality. The fact that certain netropsin conjugates capable of inducing DNA damage at specific sequences exhibit potent antitumor activities (e.g., netropsin–nitrogen mustard conjugates) shows that this DNA-targeting strategy has the potential to yield new and efficient antitumor agents. There is good reason to believe that netropsin and distamycin will continue to inspire the development of new anticancer agents (*294–297*). However, it is important to bear in mind that at the present day no-one can certify that chemotherapeutic selectivity will be improved by virtue of an increase in sequence selectivity.

In the many examples presented in this review, netropsin and distamycin are almost always used to deliver cytotoxic drugs to specific sequences in DNA, but they can also be used to deliver DNA to cells. Gene transfer with netropsin–lipid conjugates has been attempted (*298*). Moreover, netropsin and distamycin analogues can also be used independently of their capacity to bind to AT-rich DNA sequences. For example, sulfonated and phosphonated ureido dimers of netropsin such as compound 90, containing six sulfonic acid units, and the phosphonic acid-containing ligand 91 represent a new class of surface acting antiviral agents. Their mechanism of action apparently does not require binding to DNA but may involve the disruption of the virus attachment to CD4+-susceptible cells via an effect on CD4-gp120 interactions (*299*).

Although there are a few netropsin conjugates under clinical or preclinical evaluation, medicinal chemists still have much to do to design new clinically useful drugs endowed with the aptitude for reading any given DNA sequence and inducing the required DNA modification to obtain the desired biological response. Computer-based methods will increasingly serve to delineate more precisely the molecular rules that govern drug–DNA recognition. The increasing evidence that DNA binding proteins such as topoisomerases and transcription factors mediate the effects of drugs suggests that in addition to elucidating the structures of drug/DNA complexes it will be necessary to examine in detail the effects of drugs on



protein/DNA complexes. Close collaboration betwee medicinal chemists and molecular biologists will b necessary if we are to gain an improved understandir of the mechanisms whereby antitumor drugs affect th function of particular oncogenes.

Over the past 15 years, research on small molecul acting on nucleic acids has not only led to therapeutical useful drugs but also provided an invaluable source structural and biological information on nucleic acid The recent discovery of new tumor active compound such as those based on netropsin and distamycin reporte here, has perhaps restored small molecules to the for front of cancer therapy. It is still premature to state tha netropsin and distamycin derivatives will provide ne generations of sequence-specific antitumor drugs. Hov ever, the results obtained thus far are suggestive an promising. They augur exciting developments for yea to come.

ACKNOWLEDGMENT

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